ISSN 0704-3716

Canadian Translation of Fisheries and Aquatic Sciences

No. 5380

Effect of isopropanol (IPA) - preservation on industrial fish

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Original title: Verknad av isopropanol (IPA) - konservering pa industrifisk

In: Fiskeridirektoratet. Rapporter og meldinger 8/86

Original language: Norwegian

Available from:
Canada Institute for Scientific and Technical Information
National Research Council
Ottawa, Ontario, Canada K1A 0S2

1988

35 typescript pages

90-01556/

Secretary of State / Secrétariat d'État

MULTILINGUAL SERVICES DIVISION – DIVISION DES SERVICES MULTILINGUES

TRANSLATION BUREAU BUREAU DES TRADUCTIONS

LIBRARY IDENTIFICATION – FICHE SIGNALÉTIQUE

Translated from - Traduction de	Into - En
Norwegian	English

Author - Auteur

Einar Sola and Terje Ødegård

Title in English or French - Titre anglais ou français

Effect of Isopropanol (IPA) preservation on industrial fish

Title in foreign language (Transliterate foreign characters)
Titre en langue étrangère (Transcrire en caractères romains)

Verknad av isopropanol (IPA)-konservering pa industrifisk

Reference in foreign language (Name of book or publication) in full, transliterate foreign characters.
Référence en langue étrangère (Nom du livre ou publication), au complet, transcrire en caractères romains.

Fiskeridirektoratet, Rapporter og meldinger. Nr. 8/86

Reference in English or French - Référence en anglais ou français

Fisheries Directorate, Reports and Notes, No. 8/86

Publisher - Éditeur	DATE OF PUBLICATION / DATE DE PUBLICATION			Page Numbers in original / Numéros des pages dans l'original
Fiskeridirektoratet (Norway)	Year / Année	Volume	Issue No. / Numéro	3 - 29
Place of Publication / Lieu de publication				Number of typed pages / Nombre de pages dactylographiées
Norway	86		8	31

Requesting Department / Ministère-Client: DFO

Translation Bureau No. / Notre dossier nº: 2792057

Branch or Division / Direction ou Division: IPB

Translator (Initials) / Traducteur (Initiales): BP

Person requesting / Demandé par: Nancy Johnson

Your Number / Votre dossier nº:

Date of Request / Date de la demande: Feb. 26. 88

Canada

SEC 5-111 (84-10)

Secretary of State | Secrétariat d'État

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Client's No.–N° du client	Department – Ministère	Division/Branch – Division/Direction	City – Ville
	DFO	IPB	
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3287272	Norwegian	LT	

Directorate of Fisheries

Reports and Notes No. 8/86

Sola, Einar and Odegaard, Terje :

EFFECT OF ISOPROPANOL (IPA) - PRESERVATION ON INDUSTRIAL FISH

(Study carried out in 1985)

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SEC 5-25 (Rev. 82/11)

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CONCLUSION

The trial shows that isopropanol, when used as a preservation agent, has good properties, which was not known earlier. The results indicate that surprisingly low concentrations had a good preserving effect. Five to ten % IPA in raw material is sufficient to control bacterial growth during transport from fishing grounds to factory at a normal temperature, for about 10 days. Analyses of total volatile N, NH_3, TMA and TMAO indicate also that an addition of IPA effectively inhibits decomposition processes.

With regard to long-term storage at higher IPA concentrations, it is more difficult to evaluate the raw material quality in a non-ambiguous manner, because several uncertain factors have to be considered. The bacteriological and chemical analyses in this trial indicate that raw material preserved with 37 - 55 g% IPA in fl.w. kept very well for 5 months. Analyses of tot.fl.N and TMA indicate that some form of decomposition took place in raw material stored in 18 - 29 g% IPA in fl.w. . However, it is not known whether this process had anything to do with the nutritive value of a finished product.

As for fresh raw material, the amino acid distribution did not change in samples with 37 - 55 g% IPA in fl.w., while the contents of lysine, arginine and histidine decreased in samples with 18 - 29 g% IPA in fl.w.. What actually happens to protein when the raw material is stored in high concentrations of IPA is not known and must be studied further.
With regard to nutritional considerations, it has to be pointed out that the lysine content in the protein was not reduced in the two samples with the highest IPA concentrations even after a storage period of 5 months.

The fat analyses show that the samples with 29 - 55 g% IPA fl.w. were much more exposed to the risk of becoming rancid than the two other samples. The study did not explain the reason why oxidation processes were different in samples with high and those with low IPA concentrations, but it may be of interest to carry out comparisons between amino acid distributions in the protein. A high IPA concentration can block a possible antioxidant effect of amino acids. The analytical results obtained with free fatty acids indicate that isopropanol decreased the decomposition of the raw material, which is attributed to enzymatic activity.

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Client's No.–N° du client	Department – Ministère	Division/Branch – Division/Direction	City – Ville
	DFO	IPB	Ottawa
Bureau No.–N° du bureau	**Language – Langue**	**Translator (Initials) – Traducteur (Initiales)**	
2792057	Norwegian	BP	20/4-88

EFFECT OF ISOPROPANOL (IPA) PRESERVATION ON INDUSTRIAL FISH

(Study carried out in 1985.)

Reports and Notes No. 8/86.

By Einar Sola and Terje Ødegård

II INTRODUCTION

Preservation of fish raw material using isopropanol (IPA) is relevant in connection with processes which produce fish protein concentrate (FPC) for food purposes, or for animal and fish feed by extraction using IPA. Such preservation can be utilized for all types of fish which are appropriate for such purposes ("industrial raw material"). It is known that by using IPA preservation the raw material can be stored for 4-6 months, maybe longer, without noticeable reduction in the value or properties of the product. Bacterial decomposition (rotting) of the raw material appears to stop, even at rather low IPA

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concentrations, while a certain enzymatic decomposition (autolysis) appears to take place somewhat dependent on temperature, IPA concentration and type of raw material. This need not lead to complications in the process or noticable reduction in product quality.

The relationship between bacterial decomposition, temperature and IPA concentration is not well known. The same applies to the enzymatic decomposition which takes place during autolysis. The purpose of the project that is the subject of this report was to obtain a better understanding of the conditions mentioned.

Bait-containing summer capelin was selected as raw material because the content of bait strengthens the autolysis and the raw material is poorly suited to storage. Other chemical preservation methods used for such raw material have not succeeded in producing meal of sufficiently high quality for the most economical feeding purposes. Such meal has, so far, only been produced from unspoiled fresh or iced summer capelin and that limits, to a great extent, the storage period between catch and production. Feeding experiments have shown that IPA-preserved capelin produce meal of a high quality for all feeding purposes, even after storage of as much as three to four months, and maybe longer. Such storage can, capacity-wise, be achieved by increasing the tank volume in step 1 in the HEFI/EFM process, and the storage will then be included as part of the process.

The perspectives that this opens up with regard to catch and operations are almost obvious and will not be discussed here.

There are two alternatives which stand out for the landing of the catch:

1) Icing on the fishing grounds immediately after catching (as is done now), bringing ashore and preserving the raw material using an IPA strength as for production step 1 is sufficient for very long raw material storage at normal outdoor temperatures.

2) Preservation on the fishing grounds using amounts of IPA which will produce satisfactory raw material quality at time of landing. During unloading of the catch, the IPA is then adjusted upwards to full strength for further storage and production. This alternative requires fishing vessels to bring along the necessary quantity of IPA for short-term preservation, but both the transportation and the processing on board will be far less complicated than when icing/cooling. IPA preservation will also be cheaper to use, since IPA is retrieved during production on shore, while icing involves consumption of ice (which adds water to the raw material) which, depending on drainage possibilities on shore, may increase the production cost in addition to the expenses for ice. Any IPA preservation on board will require watertight and closed raw material rooms. However, this should be no problem since that is normal when icing/cooling.

IPA preservation on board fishing vessels will only become relevant in connection with FPC production based on IPA as, for instance, in the HEFI/EFM process shown schematically in Figure 1.

Concentrated IPA is combustible, but its combustibility decreases sharply when diluted with water (see Figure 2). Even 100% IPA must be heated to 12°C to burn (combust), while IPA below, for example, about 40g% (ca. 47 v%) will not be combustible (flammable) at all at normal temperatures (up to ca. 25°C). IPA can easily be diluted with water and an IPA fire can therefore be swiftly and effectively put out with water. IPA vapour is explosive when mixed with air, but only within relatively narrow margins (2 to 12 vol%). The danger area between 2 and 12 vol% does not occur at normal temperature above an IPA liquid of less than 40 g%. Since there is never a question of preservation with stronger than 20 % IPA in a volatile liquid in the raw material on board - probably far weaker - there is no danger of fire or explosion in the load of fish. The fishing vessel must, however, bring along IPA which, for reason of volume and space, must be as strong as possible. Most suitable would probably be to bring along reclaimed IPA (ca. 94 g% = 96 v%) from the process ashore. This will, under certain conditions, be both a fire and explosion hazard, but no worse than ordinary diesel fuel for the propulsion of the vessel. In addition, it is far easier to put out an IPA fire with water.

Based on this, there should be no reason for great objections to the use of IPA preservation on board fishing vessels. To reduce the amount of IPA needed on board as far as possible, it is, however, important to clarify the IPA strength which is needed to effectively retard bacterial activity while bringing the raw material ashore.

The experiment reported here therefore concerns IPA preservation of summer capelin, which was begun immediately after the catch and maintained without a break all the way to production. In order to preserve raw material on the fishing grounds, it is important to find the optimal preservation capability with minimal use of IPA. During factory storage, the amount of IPA will not be a limiting factor, such as on board vessels. Nevertheless, it is important to discover any negative aspects during long-term storage of raw material with a high IPA concentration. Emphasis was placed on discovering the effect of IPA preservation on bacterial activity, autolysis, fat quality and protein quality.

Figure 1.

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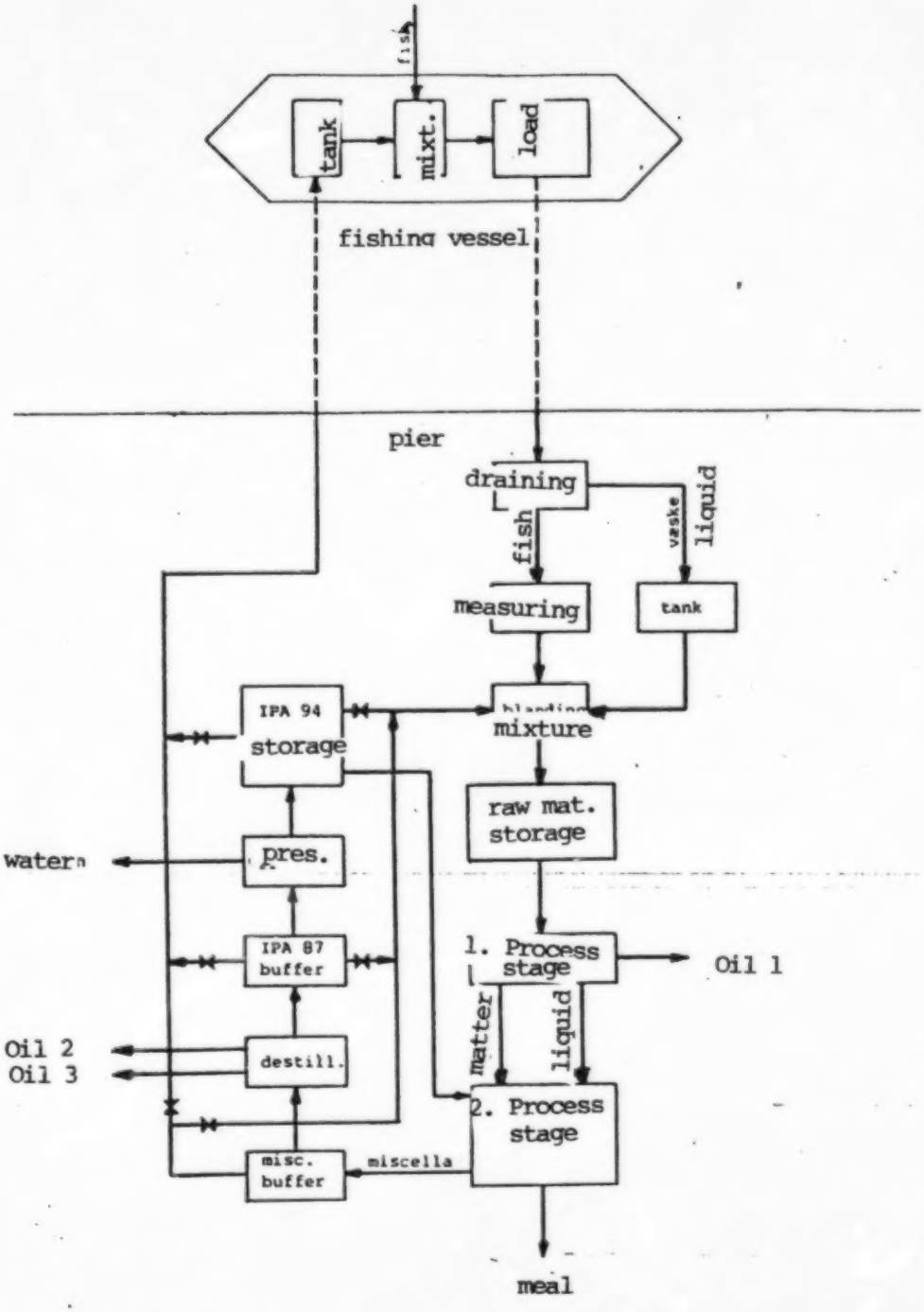

Figure 1.

EFM Process using IPA preservation during catch.

Figure 2.

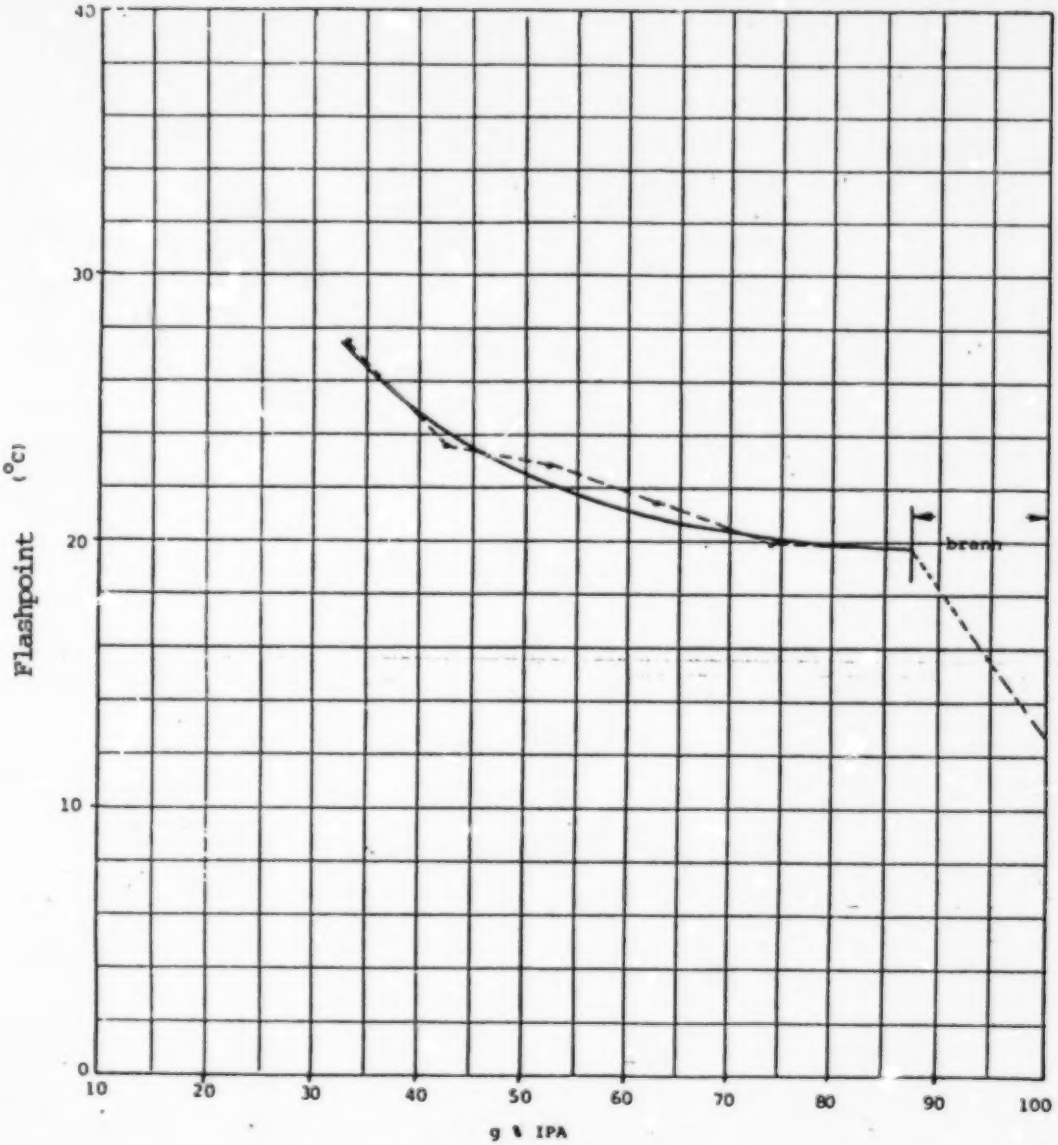

Figure 2.
Flashpoint (^{o}C) - g% IPA

III PRACTICAL IMPLEMENTATION

Seven samples of summer capelin were preserved with varying amounts of 100% isopropanol (IPA) immediately after being caught. One sample was without preservation.

Table 1.

Raw material analysis.

Dry matter	35.7%
Fat	21.7%
FFA *	14.0%
Water	64.3%

During transportation from the fishing grounds to the factory samples were taken every day and frozen for later analysis. The temperature was about 5-6°C during transportation. After a further one month storage at 8°C, samples were again drawn for analysis. The capelin which had been preserved in 6.1% and 12.2% IPA was not analyzed again after this, as it appeared that a strong deterioration of quality had taken place. The rest of the samples were stored for a further 4 months, when the experiment was terminated.

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* Fft in original. Probably typo for Ffs = Free Fatty Acid. TR.

Table 2.

IPA strength in sample material

FISH		IPA						
Kg Fish	Kg Liquid	L	Kg	kg preserv. mass	g% IPA of mass	Kg dm in mass	% dm of mass	IPA strength g% of vol.liq.
1,5	0,96	1,5	1,19	2,69	44,0	0,54	20,1	55,1
2,7	1,74	1,3	1,03	3,73	27,6	0,96	25,7	37,1
7,5	4,82	2,5	1,98	9,48	20,9	2,68	28,3	29,1
8,0	5,14	2,0	1,58	9,58	16,5	2,86	29,9	23,5
8,5	5,47	1,5	1,19	9,69	12,3	3,03	31,3	17,9
3,6	2,31	0,4	0,32	3,92	8,2	1,29	32,9	12,2
3,8	2,44	0,2	0,16	3,96	4,0	1,36	34,3	6,1
4,0	2,57	0,0	0,0	4,00	0,0			0

Specific gravity of IPA = 0.79

dm = dry matter

$$\text{IPA strength} = \frac{\frac{a}{(100 - dm)}}{100}$$

a = g% IPA in sample.

The IPA concentrations are approximate values, since the dosage was done using a litre measure. Extra diluted IPA was added to the two samples with the least IPA to cover the raw material.

In order to obtain more exact data on preservation with low concentrations of IPA, an additional experiment was carried out in the laboratory. The starting point was frozen capelin brought from the fishing grounds. This raw material was stored for a total of 20 days and analytical samples were taken throughout that time. For the first four days the temperature was at 12-15°C, later it was adjusted to about 9°C.

10.

For this method, the time on the fishing grounds was brief, since the whole catch was caught in one haul on 18.9.84. It took only four days from the time the fish was caught until it was delivered, which is a rather short period to obtain satisfactory records of the decomposition processes. The bait content in the capelin was very low (ca. 5%). This also plays a part in reducing the result of any positive preservation effect, most importantly on the samples with low concentrations of IPA, which were only to be stored for short periods of time.

11.

IV RESULTS

A. VOLATILE NITROGEN COMPOUNDS IN THE FIELD EXPERIMENT.

Figure 3 shows the development of volatile nitrogen compounds in the raw material during the first days of storage. The method of procedure for the preparation and analysis was the same as that utilized by the Central Laboratory (Sentrallaboratoriet) for inspection of raw material delivered

to the factories. When sampling for analysis, trichlor acetic acid (TCA) was added to arrest further enzymatic activity.

After four days, total vol. N increased to about 65 mg/100 g, and TMA-N was about 32 mg/100 g in the non-preserved raw material. Over this period no increase in volatile nitrogen compounds was recorded in the preserved raw material. Only after 49 days did a certain development of tot. vol. N, TMA-N and NH_3-N take place in the raw material stored in 6.1 and 12.2 percent IPA. In the samples having more than 12.2 percent IPA, no development of volatile nitrogen compounds was found. The liquid phase is not included in any of these samples.

The method of analysis is a rapid method of determining tot. vol. N; TMA-N and NH_3-N.

Table 3 shows the development of tot. vol. N, TMA-N and TMAO-N over the first days. In this analysis, the volatile nitrogen compounds are distilled after the sample has been processed to serum.

Simultaneous analysis of TMA-N and TMAO-N provides a good picture of bacteriological decomposition, since TMA is formed by bacteria which reduce TMAO and this process begins shortly after the fish is killed.

The content of TMAO in the preserved samples appeared to

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decrease without a corresponding increase in TMA. This problem is discussed separately later. Otherwise, the results of these analyses are only a confirmation of what was found in analyses of ground-up fish. In these analyses, the liquid phase was not included (drained). 12.

Table 3

Analyses of serum, tot vol. N, TMA and TMAO as mg/100g raw material.

Days	g% IPA strength in vol.liq.	Tot. vol. N	TMA-N	TMAO-N
0	0	12,2	2,0	52,8
2	0	17,1	4,1	47,3
3	0	35,2	15,3	35,4
4	0	54,1	28,4	24,6
2	6,1	12,2	2,7	42,6
3	6,1	13,9	2,3	33,0
4	6,1	13,8	1,1	35,2
49	6,1	35,7	8,1	27,2
2	12,2	12,4	1,0	42,3
3	12,2	12,2	1,0	35,3
4	12,2	12,7	1,0	33,8
49	12,2	35,2	7,9	27,4

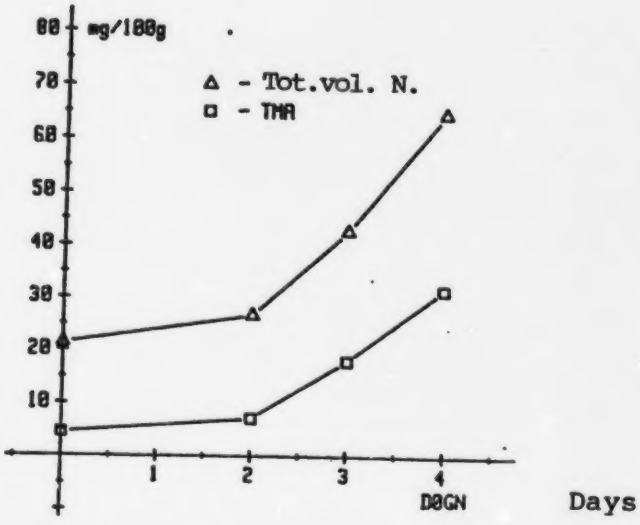

Fig. 3A. Non-preserved.

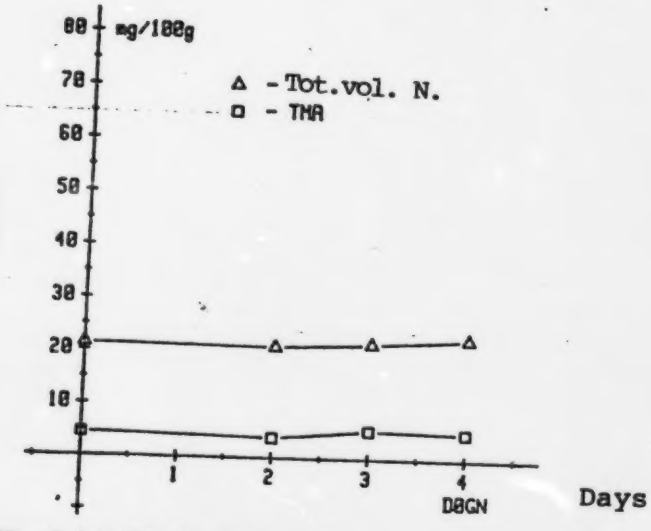

Fig. 3B. 6.1% IPA in vol. liq.

Figure 3.

Graphic presentation of development of tot. vol. N. and TMA in one non-preserved and one preserved sample. See Table 1 in Appendix A.

B. VOLATILE NITROGEN COMPOUNDS IN LABORATORY EXPERIMENT.

Unfortunately, decomposition was well on its way in the non-preserved raw material when the first sample was drawn for analysis, which was caused by the high temperature in the starting phase. Nevertheless, the results of the analyses clearly showed the development of the decomposition process. This experiment succeeded in obtaining a direct measure of bacterial development through bacteriological analysis.

It is difficult to know how the liquid phase could best be considered with regard to the chemical analyses. It is reasonable to assume that the liquid phase will change character in relation to the raw material over time, and the concentration of IPA will influence this change. An equilibrium will occur between the liquid phase and the liquid in the fish and this will be an equilibrium between the water soluble components in the fish and the liquid phase. To some degree in this experiment, attempts were made to keep constant the relationship between the raw material and the liquid.

Figure 4 shows the development of tot. vol. N, TMA and TMAO-N in fish in this experiment. After 12 days, the unpreserved capelin was bloated and in the process of complete dissolution, and the values for tot. vol. N and TMA emphasize that the raw material was badly spoiled. It took 20 days before any significant development of volatile nitrogen compounds in the sample

having 6.1 g% IPA occurred. In the sample with 12.2 g% IPA, there was only a moderate development of tot. vol. N and no increase in TMA.

Theoretically, a decrease in TMAO content could be expected to produce an increase in TMA and the analysis of the non-preserved sample showed such a development. However, for the preserved samples there appeared to be no direct relationship between a decrease in TMAO and an increase in TMA. After 20 days, however, the sample having 6.1 g% IPA showed a TMAO content tending towards zero, while the amount of TMA did not increase correspondingly. In the sample having 12.2 g% IPA, the TMA showed no increase at all, while TMAO-N decreased from about 50mg/100 g to about 30 mg/100 g.

15.

In order to get an overview of the content of volatile nitrogen compounds and trimethylamine oxide in the liquid phase, it was drained and analyzed separately. One hundred grammes of liquid was weighed and analyzed in the usual manner (Table 4). Attempts were then made to convert the amount tot.vol N, TMA-N and TMAO-N per 100 g liquid to mg/100 g fish according to the formula:

$$\frac{a \cdot x}{b} = y$$

where a = weight of liquid
b = weight of fish
x = analytical value mg/100 g liquid
y = calculated value of mg/100 g fish

See Figures 4. B and C.

Table 4.

Analyses of liquid phase in "lab. experiment".

TMAO-N and volatile N comp. mg/100 g liquid Converted to mg/100 g raw mat.

Day	G% IPA in vol.liq.	Tot. vol N	TMA-N	TMAO-N	Tot. vol N	TMA-N	TMAO-N
4	6,1	9,9	1,5	29,8	3,8	0,6	11,2
5	6,1	15,2	1,5	35,6	5,8	0,6	13,7
6	6,1	26,5	4,3	39,4	10,2	1,7	15,2
12	6,1	29,0	2,8	36,5	11,2	1,1	14,0
20	6,1	127,1	42,7	0,0	48,9	16,4	0,0
4	12,2	9,2	2,8	35,2	3,4	1,0	13,0
5	12,2	12,4	2,8	38,2	4,6	1,0	14,1
6	12,2	17,6	1,5	44,4	6,5	0,6	16,4
12	12,2	23,4	2,3	46,5	8,7	0,9	17,2
20	12,2	29,6	2,9	47,7	11,0	1,1	17,6

It would not be correct to place too great an emphasis on the exact values and individual results in the liquid analysis because there are too many uncertainties in the method of procedure. However, together with the other results, the liquid analysis provided valuable information regarding the processes in the preserved samples.

In the Table for total amounts of TMAO-N and volatile nitrogen compounds, the converted values from the liquid analyses were summed with the values from the serum analysis.

Figure 4. shows that the sample having 6.1 g% IPA was badly spoiled after 20 days storage. It appears that the process was gradual for the first 12 days, but tot.vol. N and TMA developed more spontaneously thereafter. In the sample having 12.2 g% IPA, the amounts of TMA and TMAO did not change significantly, indicating little bacterial activity. The content of tot.vol. N increased steadily and reached about 41 mg/100 g after 20 days. With this increase in tot. vol. N, a far higher value could have been expected than was found. This problem will be discussed later.

Figure 4.

Development of tot. vol. N, TMA and TMAO-N in samples in the lab. experiment.

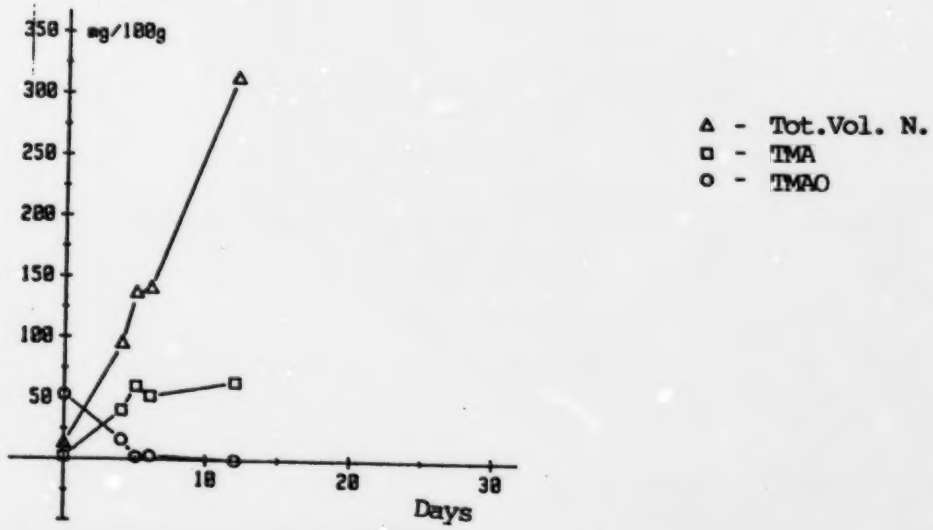

Fig. 4A. 0% IPA (Table 2, Appendix A).

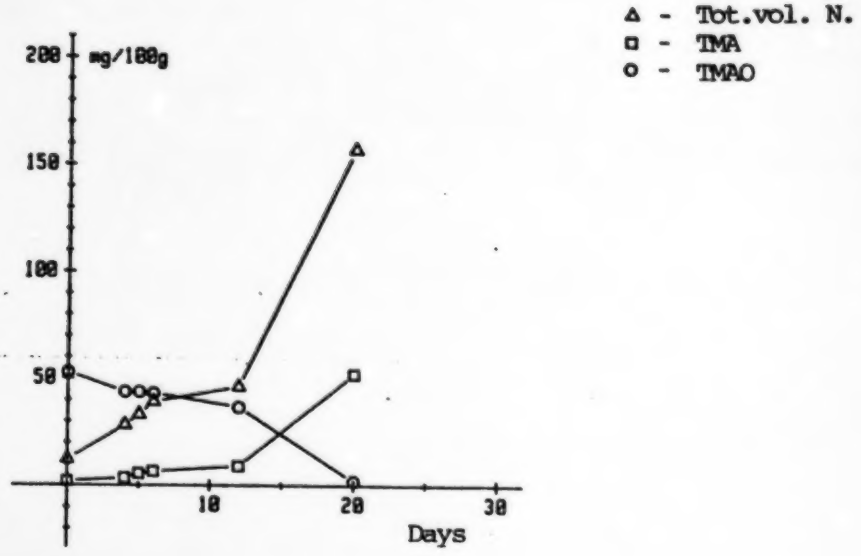

Figure 4B. 6.1% IPA (Table 3, Appendix A).

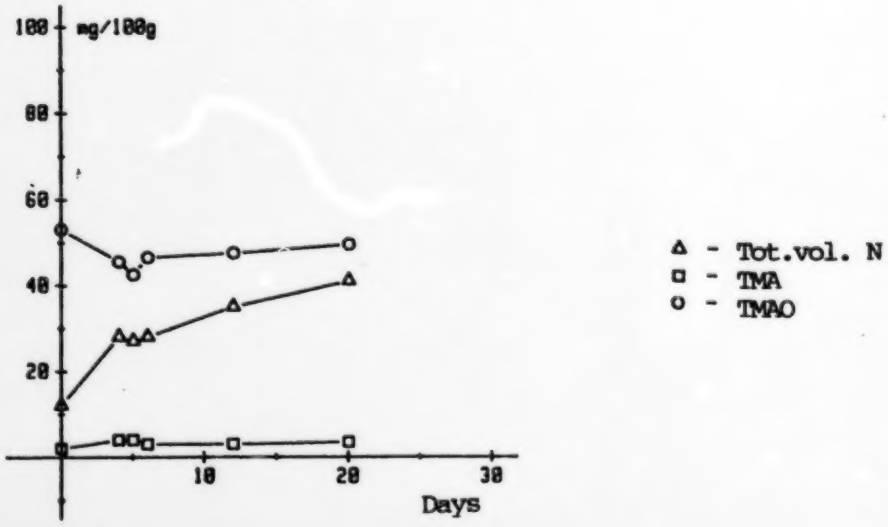

Figure 4C. 12.2% IPA (Table 3, Appendix A).

C. BACTERIOLOGICAL ANALYSIS

Figure 5 shows total number of bacteria in samples having 0, 6.1 and 12.2 g% IPA.

Figure 5 shows that bacterial activity in the non-preserved sample was already great after four days and during the next 2 - 3 days the number of bacteria increased sharply. Only after eight days was there an indication of bacteria in the sample having 6.1 g% IPA, and from 13 to 20 days the number of bacteria increased sharply. In the sample having 12.2 g% IPA, no bacteria were found even after 20 days. The development which was found in the bacterial analyses corresponded well with the results of the chemical analyses for TMA and TMAO.

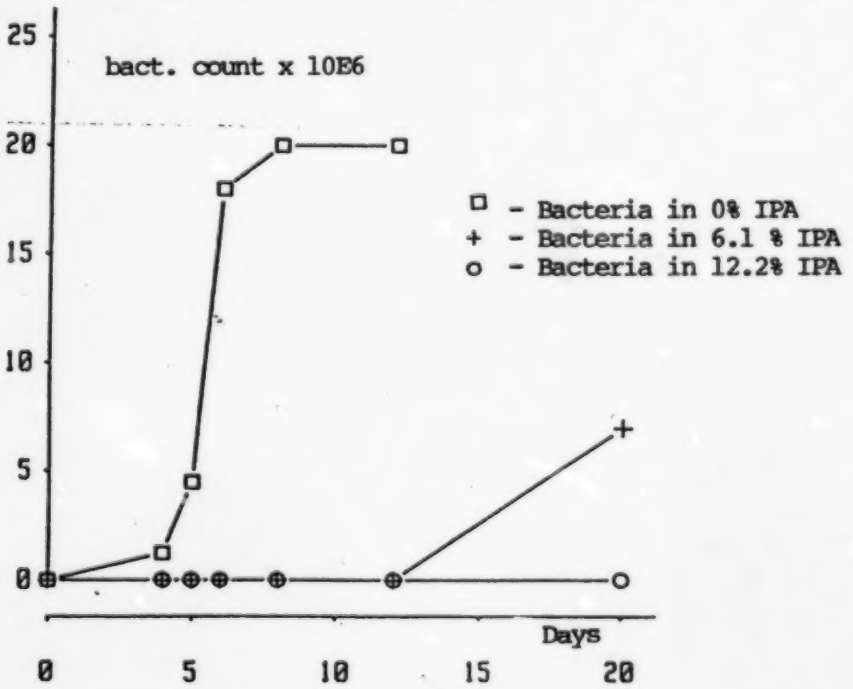

Figure 5.

Total number of bacteria in samples having 0, 6.1 and 12.2 g% IPA. (Table 4 in Appendix A).

D. RESULTS AFTER 5 MONTHS - IPA PRESERVATION OF FRESH CAPELIN.

a. Volatile nitrogen compounds.

The preservation was concluded after five months. Samples analyzed were then found to have a strength of 17.9%, 29.1%, 37.1 % and 55.1% IPA.

In samples having 37.1 and 55.1 g% IPA the fish were whole, stiff and totally without bloating of the belly. The storage liquid was clear, but a little yellow. The fish in the samples having 17.9 and 29.1% IPA were soft and some individuals had a tendency to dissolving belly. The liquid phase was grey and clearly contained an amount of dry matter. In hopes of being able to obtain an impression of the degree of autolysis, the liquid was analyzed for dry matter, protein and fat (Table 5). This was done for the lack of a better available method.

Table 5.

Analysis of dry matter, protein and fat in the liquid phase.

g% IPA in vol.liq.	% dry matter	% protein*	% fat
17,9	11,8	47,7	3,5
29,1	10,6	45,7	4,4
37,1	2,7	-	0,8
55,1	2,0	-	0,6

*On the basis of tot. dry matter.

The final analyses showed that the contents of tot. vol. N and TMA (Table 6) were very high in the raw material which had been stored in 17.9 and 29.1 g% IPA. The amount of tot. vol. N was far above the limits of 130 mg/100g set by the State Inspection Board for poor quality, but, nevertheless, it appeared that there was still some TMAO left. The samples having 37.1 and 55.1 g% IPA clearly differed from the other two, since the increase in tot. vol. N was very moderate and TMA did not increase at all, while TMAO-N decreased to about 30 mg/100g.

20.

Table 6.

Analysis of capelin stored in IPA for 5 months.

Analysis of fish				Analysis of liquid converted to mg/100 g raw material		
g% IPA in vol.liq.	Tot. vol. N	TMA-N	TMAO-N	Tot. vol. N	TMA-N	TMAO-N
17,9	150,7	41,4	3,7	31,9	8,8	1,6
29,1	133,7	39,4	5,4	45,2	13,6	3,6
37,1	15,6	1,8	19,0	3,9	0,6	10,8
55,1	19,0	2,4	12,9	11,5	1,8	21,7

Total amount tot. vol. N, TMA-N and TMAO-N as mg/100g raw material corrected for volatile N-compound and TMAO-N in the liquid phase.

g% IPA in vol.liq.	Tot. vol. N	TMA-N	TMAO-N
17,9	182,6	50,2	5,3
29,1	178,9	53,0	8,6
37,1	19,5	2,4	29,9
55,1	30,5	3,8	34,6

b. Bacteriological examination

Bacteriological examination (Table 7) showed that the total number of live bacteria was below 100 in all four samples. The number of bacteria, absence of rotting smell and the fine consistency of the raw material indicated that no decay, in the ordinary sense, had taken place. The high values for volatile nitrogen compounds in the two samples with the lowest IPA concentrations may be due to a pure dissolution effect on connective tissues and protein. In the sample having 12.2 g% IPA an increase of tot. vol. N was also found during laboratory experiments though it could not be traced directly back to decay in the raw material.

21.

Table 7.

Bacteriological examination of raw material stored for five months in IPA

g% IPA in vol.liq.	Bacteria count.
17.9	1,000
29.1	1,000
37.1	1,000
55.1	1,000

c. Fat analyses.

The analyzed fat (Tables 8 and 9) was extracted from raw material using chloroform, and the fat content was measured at

about 22 percent having 5.9 percent free fatty acids (FFA) in fresh raw material (Table 9). After five months, a certain increase in FFA took place in raw material stored in 17.9% and 29.1% IPA. The increase in FFA was not dramatic, but quite evident in the sample having 29.1% IPA. The decrease in FFA in the samples with 37.1 g% and 55.1 g% IPA was due to the fatty acids having converted to liquid phase. Since free fatty acids are splitting products from enzymatic decomposition, the FFA content tells us something about the enzymatic activity in the raw material.

Peroxide number and anisidine number are measures of oxidization of fat; anisidine number is an expression of the amount of aldehyde in the fat. Upper acceptable limit for the ansidine number in fish oil is set at about 20. It is evident that the raw material which was stored in 37.1 and 55.1 g% IPA had been far more exposed to rancidity than the two samples with lower IPA concentrations, but the reason for this observation was not determined by this experiment.

It is evident from Table 9 that the iodine number was not influenced by IPA concentrations, but was at about 135 for all samples.

Table 8.

Fatty acid distribution - weight % of fat in raw material stored in IPA for 5 months, the 0-sample was stored frozen.

Fatty Acids	IPA strength 17,9	29,1	37,1	55,1	0
C14 : 0	8,2	8,7	9,3	8,2	8,7
C16 : 1	8,0	8,4	8,9	8,6	9,0
C16 : 0	11,6	12,6	13,3	12,1	12,1
C18 : 2+3	2,5	2,7	2,8	3,0	2,4
C18 : 1	10,3	11,0	11,7	10,4	11,7
C18 : 1	2,3	2,5	2,6	2,3	2,5
C18 : 0	1,2	1,3	1,4	1,3	1,2
C20 : 5	8,7	8,3	8,1	8,4	8,6
C20 : 1	19,7	19,6	19,8	20,2	19,5
C22 : 6	6,7	5,7	5,1	5,7	5,7
C22 : 5	0,7	0,6	0,6	0,5	0,6
C22 : 1	20,1	17,9	15,9	18,9	17,6
C24 : 1	0,1	0,8	0,6	0,5	0,5

The table indicates that no significant change occurred in the distribution of fatty acid in the raw material

Table 9.

Fat analyses of raw material stored in IPA for 5 months; the 0-sample was stored frozen.

	Solid Phase					Liquid Phase	
IPA Strength	% fat	FFA	Anisi-dine	Perox-ide	Iodine number	% fat	FFA
0	21,7	5,9					
17,9	23,8	6,5	3,0	0,7	134,8	3,5	11,5
29,1	20,5	14,5	3,3	1,1	133,7	4,4	15,4
37,1	23,0	4,5	32,9	24,7	134,3	0,8	53,5
55,1	22,0	2,7	23,9	13,8	135,9	0,6	57,5

d. Amino acid distribution in proteins.

Table 10 shows loss of the three amino acids lysine, histidine and arginine in the samples having 17.1 g% and 29.1 g% IPA, while in the samples having 37.1 g% and 55.1 g% IPA the amount of the three amino acids was at the same level as in the 0-sample. Leucine appeared to have been enriched in the two samples having the highest IPA concentrations and, for the rest, the content of the other amino acids showed only minor variations with the IPA concentration.

Based on the distribution of amino acids in the protein in the 0-sample, nothing in the results of the analyses indicated that preservation with 37.1 g% and 55.1 g% IPA decreased protein quality. The total amount of protein also did not change in the samples having 37.1 g% and 55.1 g% IPA, while the other two samples showed a decrease in protein of about 10 percent. Previous analyses showed that the liquid phase in the two samples having the lowest IPA concentrations contained some protein, but whether this compensated for the whole decrease of protein in the raw material is unknown.

The protein in the liquid phase was not analyzed for amino acid distribution, something which perhaps could have explained the loss of lysine, histidine and arginine in samples having 17.9 g% and 29.1 g% IPA. It is possible that the three amino acids could have converted to the liquid phase without being destroyed or decomposed in one way or another.

Table 10.

Distribution of amino acids in protein of drained raw material stored in IPA for 5 months - 0-sample was stored frozen.

IPA strength	Amino acids mg/g protein									
	Lys	His	Arg	Leu	Val	Thr	Met	Ile	Tyr	Phe
17,9	62,4	15,9	31,3	74,7	47,1	47,8	27,6	40,7	29,5	35,5
29,1	64,5	18,7	35,0	75,6	46,9	47,2	27,8	40,6	30,5	37,3
37,1	85,3	20,7	54,3	81,2	46,7	47,7	29,2	40,5	36,5	39,9
55,1	84,0	21,0	54,0	81,8	45,9	47,4	28,8	40,3	36,7	39,2
0	85,1	20,8	60,6	71,5	50,0	47,8	28,5	36,4	36,9	39,3
	Asp	Ser	Glu	Gly	Ala	Cys	Tau	Tot. prot.		
17,9	86,1	38,8	138,7	59,0	62,4	7,5	10,7	61,2		
29,1	85,9	38,9	137,1	58,8	61,2	6,2	10,4	61,5		
37,1	98,0	44,6	143,9	53,4	57,4	8,9	4,7	70,7		
55,1	98,2	43,9	145,3	51,8	56,6	6,9	4,7	70,1		
0	97,9	44,9	145,9	58,1	63,1		10,2	72,6		

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APPENDIX A

Table 1.

Analyses of ground-up drained capelin;

tot. vol. N, TMA and NH_3-N as mg/100 g raw material.

Days	g% IPA strength in vol.liq.	Tot vol.N	TMA-N	NH_3-N
0	0	21,3	4,5	16,8
2	0	26,6	7,1	19,5
3	0	42,6	18,0	24,6
4	0	64,6	31,5	33,1
2	6,1	20,9	3,8	17,1
3	6,1	21,6	5,3	16,3
4	6,1	22,7	5,0	17,7
49	6,1	49,9	11,6	38,3
2	12,2	22,7	4,3	18,4
3	12,2	22,0	5,3	16,7
4	12,2	21,6	5,7	15,9
49	12,2	39,7	8,3	31,4
2	17,9	26,3	5,7	20,6
3	17,9	25,9	6,4	19,5
4	17,9	28,0	7,1	20,9
49	17,9	27,5	5,1	22,4
49	23,9	21,7	2,3	19,4
49	29,1	24,6	2,5	22,1
49	37,1	20,3	1,5	18,8
49	55,1	25,9	2,2	23,7

APPENDIX A

Table 2.

Analysis of serum, "lab. experiment". Development of tot. vol. N, TMA and TMAO in the fish, mg/100 g raw material.

Days	%IPA in vol.liq.	Tot.vol N	TMA-N	TMAO-N
0	0	12,2	2,0	52,8
4	0	94,3	40,4	16,5
5	0	135,6	60,4	2,0
6	0	139,2	52,8	2,5
12	0	312,2	64,8	0,0
4	6,1	24,7	3,0	32,1
5	6,1	27,2	4,8	29,9
6	6,1	29,4	5,8	26,7
12	6,1	34,5	7,6	22,7
20	6,1	107,8	35,7	1,9
4	12,2	24,8	2,8	32,4
5	12,2	22,3	2,8	28,3
6	12,2	21,4	2,3	30,2
12	12,2	26,2	2,1	30,2
20	12,2	29,6	2,3	31,8

APPENDIX A

TABLE 3.

Total amount of TMAO-N and volatile N. compounds in the raw material during lab. experiments. Corrected for TMAO-N and vol.N compounds dissolved in the liquid phase (see Chapter IV. B).

Days	g %IPA in vol.liq.	Tot.vol N	TMA-N	TMAO-N
4	6,1	28,5	3,6	43,3
5	6,1	33,0	5,4	43,6
6	6,1	39,6	6,5	42,9
12	6,1	45,7	8,7	36,7
20	6,1	156,7	52,1	1,9
4	12,2	28,2	3,8	45,4
5	12,2	26,9	3,8	42,4
6	12,2	27,9	2,9	46,6
12	12,2	34,9	3,0	47,4
20	12,2	40,6	3,4	49,4

APPENDIX A

Table 4

Bacteriological examination of raw material in the lab. experiment.

Days	g %IPA in vol.liq.	Bact. count
4	0	$1,23 \times 10^6$
5	0	$4,50 \times 10^6$
6	0	$18,00 \times 10^6$
8	0	$20,00 \times 10^6$
12	0	$20,00 \times 10^6$
4	6,1	1000
5	6,1	1000
6	6,1	3000
8	6,1	25000
12	6,1	1000
20	6,1	$7,00 \times 10^6$
4	12,2	1000
5	12,2	1000
6	12,2	1000
8	12,2	1000
12	12,2	1000
20	12,2	1000

APPENDIX B

METHODS OF ANALYSIS.

Total volatile nitrogen, trimethylamine, ammonia and trimethylamino oxide, was determined according to method described by Hjorth-Hansen and Bakken (1947).

Bacteriological examination: Total number of live bacteria was determined according to Central Laboratory (Sentrallaboratoriet) method no. 41.

Fat was analyzed according to Sentrallaboratoriet method No. 36, but chloroform was used as an extractant.

Peroxide number was determined according to Sentrallaboratoriet method No. 24.

Iodine number was determined according to Sentrallaboratoriet method no. 21.

Anisidine number provides a measure of rancidity, that is, an analysis of aldehyde in fat. UIPAC method ISO/TS 34/SC 11-N 94 June 1978 was used.

Distribution of fatty acid: The fatty acids were determined as methyl esters extracted using chloroform/hexane and separated on a gas chromatograph.

Amino acid distribution in protein; this analysis was carried out at the Fisheries Directorate, Institute of Nutrition (Njaa).